Gas Chromatographic/Thermal Energy Analyzer Method for *N*-Nitrosodibenzylamine in Hams Processed in Elastic Rubber Netting

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We previously described a solid-phase extraction (SPE) procedure for determining volatile nitrosamines in hams processed in elastic rubber nettings. This same procedure was found to successfully isolate *N*-nitrosodibenzylamine (NDBzA), a semivolatile nitrosamine. This nitrosamine may form as a result of the reformulated rubber now used in nettings. Reformulation became necessary because of the reported presence of *N*-nitrosodibutylamine in both the old nettings and on the exterior portion of commercial hams. After SPE, NDBzA was quantitated by using a gas chromatographic (GC) system interfaced to a nitrosamine-specific chemiluminescence detector [thermal energy analyzer (TEA)]. The GC system was equipped with a heated interface external to the TEA furnace to facilitate quantitation of NDBzA. With separation on a packed column, the method can be used to analyze 10 volatile nitrosamines and NDBzA. Repeatability of the method for NDBzA was found to be 2.1 ppb, and the coefficient of variation (CV) was 10.6%. Analysis of 18 commercial hams from 9 different producers, purchased from local retailers, indicated that 12 were positive for NDBzA (range, 2.6–128.5 ppb). NDBzA was confirmed by GC/mass spectrometry.

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Various meat and poultry products, particularly boneless hams, are processed in elastic rubber nettings. These nettings are used to hold large pieces of meat together during processing to help form the shape of the product. However, several types of chemicals used in the formulation of rubber have the ability to form nitrosamines. One type includes vulcanization agents such as zinc dithiocarbamate and thiuram sulfide derivatives. Zinc dibutyldithiocarbamate may still be present after rubber processing and capable of being nitrosated to form a potent animal carcinogen, *N*-nitrosodibutylamine (NDBA) (1). In addition, the rubber may also contain dibutylamine, both as a precursor for the synthesis of the accelerator and as a decomposition product.

Sen et al. (2) were the first to report the presence of NDBA and *N*-nitrosodiethylamine (NDEA) in Canadian nitrite-cured meats processed in elastic rubber nettings. They also found trace quantities of these same nitrosamines in the unused netting and high levels (up to 504 ppb) in the used netting. The corresponding meat samples also contained NDBA (up to 29 ppb). In the United States, the Food Safety Inspection Service (FSIS) also found high levels of NDBA in netted hams during an assessment of a new curing process for boneless hams (3). After this finding, the FSIS requested that our laboratory

develop rapid methods for the detection of nitrosamines and their precursor amines in this product type.

In 1992, Pensabene et al. (4) reported on a solid-phase extraction (SPE) method for the analysis of 10 volatile nitrosamines, and they found NDBA in netted hams in concentrations up to 50 ppb. Another study of elastic rubber nettings indicated the increased presence of the semivolatile *N*-nitrosodibenzylamine (NDBzA) and a decrease in NDBA compared with the previous 2-year period (5). Therefore, any newly developed method should be able to detect volatile nitrosamines and NDBzA. Typically, volatile nitrosamines are analyzed by distillation techniques, involving mineral oil or low temperature vacuum systems. These methods are awkward and time-consuming, and they require a substantial amount of space, equipment, and solvents. These methods are also not capable of efficiently removing NDBzA from the food matrix.

Sen et al. (6) used a lengthy and complex multiple solvent extraction technique after initial sample homogenization with acetonitrile. Two azeotropic distillations, treatment of the filtrate through an alumina column, and washing with pentane, were carried out before final elution with dichloromethane. This method was separate from that used for the volatile nitrosamines. Our SPE method, previously reported for volatile nitrosamines (4), being a nondistillation technique, offered an opportunity to also analyze for NDBzA. Here we report on the successful modification of this gas chromatography (GC)/thermal energy analysis (TEA) quantitative method and present the results of a limited study used to determine the extent of NDBzA in commercial netted boneless hams.

METHOD

Caution: *N*-Nitrosamines are potential carcinogens. Exercise care in handling these compounds.

Reagents

(a) *Celite 545, sodium sulfate, propyl gallate, dichloromethane (DCM), pentane, and ethyl ether.*—These reagents were described previously (4).

(b) *Silica gel.*—70–230 mesh (EM 7734). Prewash twice with DCM, dry 4 h in 60°C vacuum oven, and then sieve through a 70–150 mesh.

(c) *N-Nitrosodipropylamine (NDPA) internal standard solution.*—0.10 μg/ml in DCM.

(d) *GC working standard solution.*—NDPA, *N*-nitrosopiperidine (NPIP), NDBA, and NDBzA, each at a concentration of 0.10 μg/mL in DCM. These nitrosamines were either purchased or synthesized from their corresponding amines and sodium nitrite according to the general procedure published previously (7).

(e) *Hams.*—Samples were obtained from local retail outlets or producers and were analyzed without further heating. The outer ¼ in. of the ham was removed, ground through a 1/16 in. plate, and then thoroughly mixed. The comminuted sample was stored in a –20°C freezer until analyzed.

Apparatus

(a) *Mortar, pestle, chromatographic columns, tamping rod, evaporative concentrator.*—These were described previously (4).

(b) *GC/TEA system.*—Shimadzu Model GC-14A (Columbia, MD) connected to an external pyrolyzer interface controlled by a TEA Model 610R Nitrogen Converter (Woburn, MA), which in turn is interfaced to a TEA Model 502A. GC and TEA operating conditions: 1.8 m × 2.6 mm glass column packed with 5% SP-2401 DB on 100–120 mesh Supelcoport; He carrier gas, 35 mL/min. Column program: 80°C for 5 min, and then 10°C/min to 220°C. Injector, 240°C; pyrolyzer, 475°C; interface, 275°C; TEA vacuum, 1.0 mm; and a liquid nitrogen cold trap.

(c) *Mass spectrometric (MS) system.*—Hewlett-Packard Model 5971 MSD (Valley Forge, PA) interfaced to a Hewlett-Packard Model 5890A GC. Operating conditions: 30 m × 0.32 mm DB-5-MS capillary column, 1 μ phase thickness. GC splitless injector, 220°C; GC/MS interface, 300°C; MS source, 190–200°C; He carrier gas, 1.2 mL/min. Column program: 70°C for 5 min, and then 6°C/min to 220°C; MS purge on at 2 min, off at 5 min; MS vacuum 50–70 mTorr.

Determination

(a) *Sodium nitrite analysis.*—Residual sodium nitrite was determined in 10.0 g of comminuted hams by the Griess-Saltzman procedure as modified by Fiddler (8).

(b) *Solid-phase extraction (SPE).*—The details for the determination of the nitrosamines, including NDBzA, have been published previously (4). A flow diagram outlining this procedure is shown in Figure 1.

(c) *Nitrosamine determination.*—Quantitate nitrosamines as described previously (4). The minimum detectable level (signal:noise >2) for NDBA, NPIP, and NDBzA was 1.0 ppb.

(d) *Nitrosamine confirmation.*—Before MS confirmation, the sample was passed through a silica gel Sep Pack using the same solvent system described in the SPE procedure (4), then concentrated to 0.2 mL before injection into the GC/MS system. In the scan mode, scan from 29 to 240 amu at 2.3 scans/s using Mid-Mass Autotune. The nitrosamine was considered confirmed when the GC peak in the ham extracts had the same retention time as the nitrosamine standard and when the full spectrum matched (>90%) a previously run standard. The spectra were matched by algorithms built into the MSD software. A sample required a minimum of 10 ng/injection of NDBzA for confirmation.

(e) *Statistical analysis.*—Data were analyzed by the general linear model and means procedures of Statistical Analysis System PC software (9). These results were then interpreted according to methods of Snedecor and Cochran (10).

Results and Discussion

The SPE method used for the analysis of volatile nitrosamines in netted cured meat products was already demonstrated to be free of artifact formation by the addition of a large excess of an amine capable of rapid nitrosation (4). The isolation of

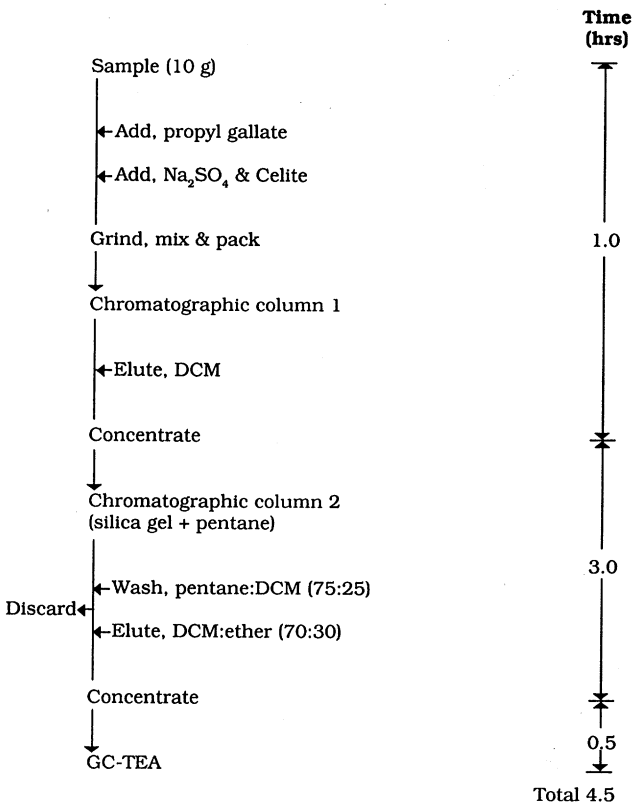

Figure 1. A schematic of the solid phase extraction procedure: apparatus consisted of 1 mortar and pestle, 2 chromatographic columns, 1 tamping rod, 2 receiving flasks, and 2 K-D evaporators and Snyder columns; chemicals were 0.25 g of propyl gallate, 25 g of Na_2SO_4, 20 g of celite, 4.0 g of silica gel, 300 mL of DCM, 112 mL of pentane, and 45 mL of ether; rate was 6–8 analyses per day per analyst.

NDBzA in ham required no modifications of this method, but we did have to change to the GC/TEA detection system because of the limited volatility of NDBzA. Sen et al. (11) were the first to use a high temperature transfer line between the GC and the TEA. We also found that a heated interface was necessary to transfer the NDBzA from the GC column directly into the TEA pyrolyzing furnace, which cleaves the N–NO bond. If the TEA is interfaced to the GC in the conventional way used for volatile nitrosamines, the NDBzA peak is so broad on a packed column that quantitation is impossible. The TEA Model 610R Nitrogen Converter, designed as an add-on piece of equipment for the TEA and used for the detection of nitrogen containing compounds, was ideal for the analysis of NDBzA. The device was used in the nitroso rather than the nitrogen mode. The heated interface then could be directly inserted into the GC, thereby preventing any cold spots that would lead to the condensation of NDBzA before it entered the TEA pyrolyzer. The GC column packing used for the separation and quantitation of NDBzA was selected based on the need to detect not only NDBzA, but also the other nitrosamines previously found in netted hams, NDBA and NPIP. Several packings were evaluated before obtaining excellent results with 5% SP-2401 DB on 100–120 Supelcoport. An example of a typical chromatogram is shown in Figure 2. This SP-2401 DB packing contains a trifluoropropylsilicone-based phase used in combination with a deactivated support that is especially recommended for the analysis of amines (12). Although a capillary column may have been suitable for this purpose, the use of a packed column permitted the injection of larger sample sizes without deterioration of column performance. This was an important aspect, given the great number of samples that needed to be analyzed.

To determine if NDBzA could be recovered with the SPE procedure, we fortified ham samples, processed without net-

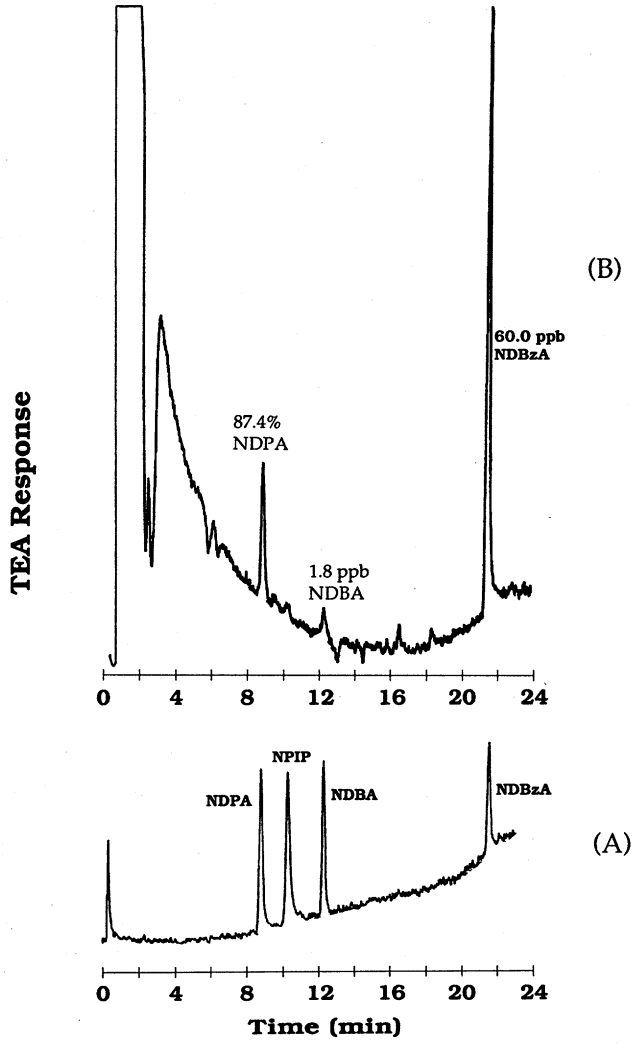

Figure 2. Chromatogram from a 9.0 μL injection of (A) nitrosamine standards and (B) a ham sample extract: NDPA, *N*-nitrosodipropylamine; NDBA, *N*-nitrosodibutylamine; NDBzA, *N*-nitrosodibenzylamine; and NPIP, *N*-nitrosopiperidine.

tings, at 5 and 10 ppb. Recovery of NDBzA was 93.0% ± 3.88M% at 5 ppb and 95.6% ± 4.58M% at 10 ppb. To determine if the same internal standard (NDPA) could also be used for NDBzA, we performed a correlation study. A significant ($P<0.05$, n = 6) correlation was found between NDPA and NDBzA. Finally, to determine the repeatability of the method with NDBzA, 8 ham samples, containing normally incurred 2–60 ppb NDBzA, were analyzed in duplicate. The repeatability was 2.1 ppb, and the coefficient of variation (CV) was 10.6% (values uncorrected for the NDPA internal standard). The internal standard, fortified at 10 ppb, gave a repeatability of 6.2%, CV of 6.9%, and an overall mean recovery for NDPA of 88.9%. On the basis of these results, we found the SPE method to be accurate and reliable for the isolation and quantitation of NDBzA from the cured meat matrix.

To determine the extent of NDBzA contamination in hams processed in elastic rubber nettings, we analyzed 18 retail samples from 9 commercial producers. The results (Table 1) were obtained from the analysis of the exterior ¼ in. portion of the hams, the site closest to the netting. Twelve of 18 samples contained NDBzA, with 10 samples having levels greater than 10 ppb. Four samples also contained traces of NDBA in addition to NDBzA, and 1 sample contained NPIP. Some samples did not contain detectable levels of either volatile nitrosamines or NDBzA (samples from companies 2–4 and 1 sample from company 5; Table 1). Sen et al. (6) were the first to report on the presence of NDBzA in cured pork products processed in reformulated elastic rubber nettings. They found negligible levels of NDBA, but up to 104 ppb NDBzA in the homogenized meat and up to 128 ppb NDBzA in the outer layer. The levels of NDBzA reported here were comparable. Our findings suggest that the majority of ham producers we tested used nettings containing reformulated rubber. Two samples, both from company 7, contained only NDBA and indicate that some ham producers may still be using the old nettings. Higher levels of these nitrosamines may be found in products from producers not covered in this limited survey.

Table 1. *N*-Nitrosamines in commercial hams

Company	*n*	Residual nitrite, ppm	Nitrosamines, ppb[a]		
			NDBzA	NDBA	Other
1	1	8.3	43.6	3.9	ND
	1	15.1	13.6	ND	ND
	1	24.2	128.5[b]	8.0	ND
2–4	3	ND–8.1	ND	ND	ND
5	1	9.3	ND	ND	ND
	1	ND	2.6	ND	ND
	1	5.9	20.1[b]	5.4	ND
	1	5.5	26.7[b]	ND	ND
6	1	ND	68.6[b]	2.1	ND
	1	2.5	25.2	ND	ND
	1	6.7	16.6	ND	ND
	1	5.6	104.5[b]	ND	ND
7	1	7.7	ND	46.7[b]	ND
	1	7.1	ND	3.1	ND
					7.4 (NPIP)[b,c]
8	1	0.7	9.4	ND	
9	1	9.1	34.4	ND	ND

[a] Outermost ¼ in. of ham: NDBzA, *N*-nitrosodibenzylamine; NDBA, *N*-nitrosodibutylamine; ND, not detected.
[b] Confirmed by mass spectrometry.
[c] NPIP, *N*-nitrosopiperidine.

Conclusion

We have reported a simple and accurate method for determining NDBzA and volatile nitrosamines in hams. Using this method, we have detected nitrosamines in cured meat products processed in elastic rubber nettings. The incidences and levels of NDBA appear to be decreasing, but now NDBzA and NPIP have been detected. One would expect the incidences of NDBzA and NPIP to increase if the recent American Meat Institute food additive petition to the U.S. Food and Drug Administration is implemented (13). This petition requests approval for single-use rubber threads in nettings for food processing, including meat and poultry, in which zinc dibenzyldithiocarbamate and pentamethylenethiuram tetrasulfide are used as vulcanization accelerators. These 2 compounds can form NDBzA and NPIP, respectively. Because some of the ham samples contained levels of NDBzA that were high by volatile nitrosamine standards, further investigations are warranted.

Acknowledgments

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